• * PALMINTRANET

Day : Monday Date: 1/31/2005

Time: 13:18:20

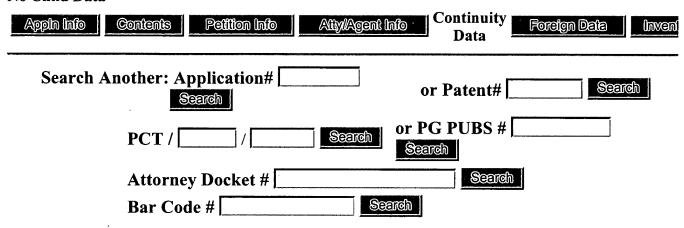
Continuity Information for 08/822033

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Application Number Information

Application Number: 09/060659 Order This

File Assignments

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Application Received: 04/15/1998

Patent Number: 6143520

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Query Request

Waiting for Response

Desc.

Status Date: 10/20/2000

Title of Invention: NOVEL EXPRESSION VECTORS AND METHODS OF USE

| Bar Code | PALM Location | Date | Charge to Loc | Name | 1 3 | Location |
|----------|------------------|------------|-----------------------|----------------------|----------------|----------|
| 09060659 | 9200 | 03/30/2004 | No Charge to Location | No Charge to Name | KASAH,EMMANUEL | |

| Info Contents Petition Info Attyl/Agent Info | Continuity Data | Foreign Data | Oto |
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coprecipitation of sFvTacKDE1 with the 40 kDa precur-

Together, these findings suggest that the absence of mature p55 at the cell surface is due to retention of the immature p40 form in the ER, as a complex with 5 sFvTacKDE1.

These results have been reproduced in two other T leukemic cell lines which express high levels of IL-2Ra. HUT102, an HTLV-1 transformed line, and the Kit225 line. The Kit225 cells are growth factor dependent and were 10 maintained on 100 units/ml IL-7. After introduction of the HIV-1 forced expression vector into these cell lines, a majority of the bulk transduced population were negative for IL-2Ra expression. By subcloning from the bulk population, homogenous negative clones were obtained 15

Efficient downregulation of IL-2Ra and reduced IL-2 responsiveness in PBMCs transduced with the sFvTacKDEL forced expression vector.

FIG. 7 shows IL-2Ra expression on PHA-activated 20 peripheral blood mononuclear cells transfected with the sFvTacKDEL forced expression vector or a control vector (1) bulk population (FIGS. 7A-C); or (2) single cell subclones (FIGS. 7D-G). Flow cytometric analysis of a bulk PBMC population transduced with the HIV-1 forced expres- 25 lentiviral vector is a lentiviral vector containing multiple sion vector showed virtually no IL-2Ra expression (FIG. 7B) in comparison to a control population transduced with an irrelevant (empty) vector HVSL3P (FIG. 7C). These cells were generated and maintained in the presence of IL-7, an single cell clones were obtained that express no detectable IL-2Ra (See FIGS. 7E and F). A thymidine incorporation assay was used to measure the IL-2 responsiveness of the IL-2Ra negative clones (FIG. 8). FIG. 8 shows IL-2 induced proliferation in peripheral blood T cell clones which are 35 positive (clone 5) or negative (clone 2) for IL-2Ra expression. These clones did not respond to low doses of IL-2 (1 unit/ml). Some proliferation was seen at doses of 10 and 100 units/ml but when compared to an IL-2R\alpha-positive clone, -10 times more IL-2 was required to achieve an equivalent

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proliferative response. Some IL-2 responsiveness was expected, even in the absence of IL-2Ra, as these cells will still express intermediate affinity receptors for IL-2. These data demonstrate functional as well as phenotypic evidence for the absence of high affinity IL-2 receptors in the IL-2R α negative cells.

We claim:

- 1. A lentiviral vector containing a gene of interest operably linked to a selectable marker gene by an internal ribosome entry site (IRES).
- 2. The lentiviral vector of claim 1, wherein the gene of interest is a gene whose expression in a mammalian cell is selected against as determined by comparing a cell transduced using a divalent vector or co-transfection with a selectable marker and said gene, with a control cell transduced using a divalent vector or co-transfection with only said selectable marker.
- 3. The lentiviral vector of claim 2, wherein the gene of interest is selected from the group consisting of a gene for HTLV-1 tax, HTLV-2 tax, an antibody and a protein that is part of a multi-tiered expression system.
- 4. The lentiviral vector of claim 1, wherein a defective lentiviral vector is used.
- 5. The lentiviral vector of claim 4, wherein the defective splice donor and splice acceptor sites.
- 6. The lentiviral vector of claim 5, wherein the lentiviral vector is an HIV viral vector.
- 7. A method of using the vector of claim 1 to obtain forced alternative T cell growth factor. By subcloning (FIG. 7B), 30 expression of the gene of interest which comprises using the vector of claim 1 to transduce a mammalian cell, culturing the transduced cell under conditions sufficed to express the selectable marker gene, and then exerting selection pressure on the transduced cell to select for that selectable marker.
 - 8. The lentiviral vector of claim 3, wherein the gene of interest is an antibody gene.
 - 9. The lentiviral vector containing two different genes linked together by an internal ribosome entry site (IRES).